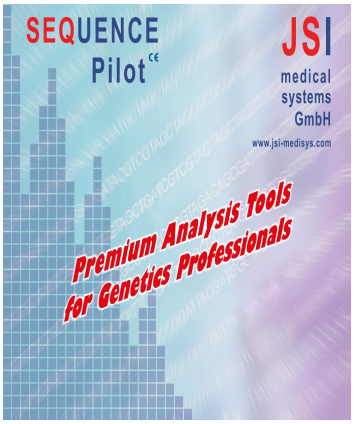
What's new

SEQUENCE Pilot CE

Version 4.0.0 and 4.01
(07/20/2012)



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1 Version 4.0.0

1.1 All modules

1.1.1 SEQUENCE Pilot screen

The **SEQUENCE Pilot** screen has a new layout.

1.2 Modules SeqNext, SeqPatient and SeqC

1.2.1 Operation Sequence/Alamut interface

An Alamut interface for mutations is available now. Therefore enter the following entry in the `lis.ini`-file, located in the `bin`-directory of your installation: Section `[SeqPilot]`: `MutationSendExtern=send to Alamut...|`http://localhost:10000/show?request=JSIDATA. There will be the context menu item *send to Alamut* available for mutations in the *Variation/Mutation table* then.

Moreover in the Alamut software the following setting has to be changed:
menu *Tools* / *Options* / register *API* / option "*enable HTTP access*" has to be activated.

1.3 Module SeqNext

1.3.1 Operations PCR Products, PCR Groups and Enrichment [master file]

These operations are not available any longer. The operations *PCR Products [master file]* and *Enrichment [master file]* were combined in the new operation *ROI [master file]* (ROI = Regions Of Interest). The operation *PCR Groups [master file*] was renamed into *ROI Groups [master file].*

Note: All enrichments and Multiplicom MASTR assays have to be defined newly for version 4.0.0!

1.3.2 Operation ROI [master file]

ROI stands for regions of interest. This operation combines the operations *PCR Products [master file*] and *Enrichments [master file]*. All regions of interest can therefore be defined in this operation. Tab *all ROI* gives an overview about all defined regions of interest. Selecting tab *add PCR* enables definition of PCR products, tab *add Enrichment* enables definition of enrichment chips and tab *add Kit* enables the definition of kits such as Multiplicom MASTR assays.

In case a single region of interest has more than 10000 bases the region is splitted into smaller regions automatically. A warning is then shown.

1.3.2.1 Tab all ROI

The *ROI List* lists all defined regions of interest (enrichments and PCR products).

In the new field *State* you can choose *new ROI*. Then only ROIs that were just added (not saved yet) will be listed.

In the new field *Amplification* you can choose an amplification. Then only ROIs where this amplification was used are listed. The amplification is shown in the column *Amplification* of the *ROI List*. For PCR products the entry is *PCR* automatically; for Enrichments and Kits it is user defined, e.g. *Roche NimbleGene, Agilent SureSelect* or *Multiplicom* (can have up to 24 characters).

Moreover in the column *Name* ROIs added on tab *add Enrichment* and on tab *add Kit* have a suffix, whereas ROIs added on tab *add PCR* have no suffix, e.g. *BRCA1-E01**-ng*** (Enrichment), *BRCA1-E01* (PCR).

The following new fields/settings can optionally be adapted for each ROI:

- *Ignored Parts*: For Ignored Parts there is no basecalling done. This function can be used in case there are parts of the ROI that are not sequenced.

  Sequence parts can be ignored in the operation *Sequence* using the new context menu item *ignore sequence part* of the combined, forward and reverse sequence.

  Sequence parts ignored in the operation *Sequence* are listed in the following way: *position1..position2*. For the numbering the amplicon count mode is used.

  To remove an ignored sequence right-click an entry and select *remove* from the context menu.

- *Ignored Sequences/Pseudogenes.*: Here sequences that should be ignored (e.g. pseudogenes) are listed. All fragments the contain the here listed sequence are not used for analysis.

  A sequence can be ignored in the operation *Sequence* in the *fragments view.* Therefore mark a sequence in the fragments view using the Ctrl key and marking the sequence with you left mouse button. Then open the context menu and select *save as ignore sequence*. These ignored sequences are listed in the field *Ignored Sequences/Pseudogenes* (operation *ROI [master file]*) automatically.

  To remove an ignored sequence right-click an entry and select *remove* from the context menu.

- *ROI Amplicon Parts*: Here amplicon parts can be defined. This is usable for all platforms now.

- *Primer (5'->3')*: Press *[Add]*. Then enter a primer sequence in 5'->3' direction into the field *Primer (5'->3')*. Only active primers will be removed! The following fields can be edited for each entered primer:
  - *Direction:*
    - *fwd:* primer is found in forward fragments
    - *rev:* primer is found in reverse fragments
    - *both:* primer is found in all fragments
  - *Modifier*:
    - *5'+rev complement*: primer is at the 5' and can also be found rev. complement.
    - *5'*: primer can be found at the 5' end only.
    - *3'+rev complement*: primer is at the 3' end and can also be found rev. complement.
    - *3'*: primer can be found at the 3' end only.
  - For normal PCRs where a primer is in the beginning and at the end of each fragment the following entry should be used: *Direction: fwd, Modifier: 5'+rev complement* and *Direction: rev, Modifiifier: 5'+rev complement*.
  - *Error rate*: here a percentage value can be entered as error rate (wrong bases, that the primer can contain). This can be done seperately for 5' and 3' end.
  - *Overlap*: Here the minimim number of primer bases the fragment must contain can be entered. Example: overlap is *3*. There must be at least *3* primer bases found in the fragment otherwise it is not trimmed. This can be done seperately for 5' and 3' end.
  - In case the primer is not in the end of the fragments an area, where the primer is searched for, can be defined as follows: ATGCTC**,10**. This entry means that the primer ATGCTC is searched for in the first **10** bases of the fragments.

1.3.2.2 Tab add PCR

Use this tab to define PCR products as done in the former operation *PCR Products [master file]*. In the new field *State new ROI* is pre-selected. Therefore only ROIs that were just added (not saved yet) will be listed in the ROI List. After saving the list will be empty. To see all defined ROIs, select the *State* blanc!

1.3.2.3 Tab add Enrichment

Note: All enrichments have to be defined newly for version 4.0.0! For orders analysed with older versions single ROIs (in the *ROI/Location table* of the operation *Sequence*) can not be recalculated.

Use this tab to define Enrichments.
The entry in *Amplification* can have up to 24 characters and should define enrichment chip, e.g. *SureSelect* or *NimbleGene*.
The entry in *Suffix* can have up to six characters. The suffix is added to the *Name* of the ROI, e.g: Suffix *ng* (for NimbleGene), ROI name is *BRCA1E01-ng*. Therefore all ROIs added on tab Enrichment have a suffix in the ROI name listed in column *Name*.

There is the setting *auto cut* available:
If you use this setting the sequence is cut to the entered values for each ROI: e.g. setting is 5' 20 and 3' 30: All ROIs are cut to 20 bases before the exon and 30 bases after the exon in case the sequences defined on the enrichment chip are longer.

The settings *auto complete, check equal, cut primer* and *combine* are not available any longer.
In the new field *State new ROI* is pre-selected. Therefore only ROIs that were just added (not saved yet) will be listed in the *ROI List*. After saving the list will be empty. To see all defined ROIs, select the *State* blanc!

All other functions are the same as in the former operation *Enrichment [master file]*.

1.3.2.4 Tab add Kit

Note: All Multiplicom Kits have to be defined newly for version 4.0.0. Orders analysed with older versions using Multiplicom kits can not be recalculated (in the operation *Joining* and in the *ROI/Location table* of the operation *Sequence*)!

Use this tab to define Kits, e.g. Multiplicom MASTR assays. The functions are the same as for Tab *add Enrichment*, here the `tsv`-file for Multiplicom Kits can be loaded instead of the files defining the enrichment.

1.3.3 Operation ROI Groups [master file]

The operation *PCR Groups [master file]* was renamed into *ROI Groups [master file]*. Here all regions of interest can be grouped.
In the field *Amplification* the amplification type, e.g. *PCR* can be selected. Only the corresponding ROIs are then listed in the table below.
Multiplicom MASTR assays are not defined in this operatian any more, they can be defined in the operation *ROI [master file]* using tab *add Kit*. The `tsv`-files and the `fas`-files including the setup data for Multiplicom MASTR assays has to locate in the folder `SeqNPart\Kits\Multiplicom` of your installation now (instead of `SeqNPart\Multiplicom`). For further details about the set up of Multiplicom MASTR assays please have a look at our **User Manual** for module **SeqNext**.

1.3.4 Operation Run

1.3.4.1 Files

- `bam`- and `sam`-files can be loaded now.
- for paired end sequencing data several groups of files can be imported now.

1.3.4.2 Settings

The settings have been changed. Profiles including special settings can be saved and selected in the field *[Profiles]*, e.g. different settings for different platforms. Moreover the fragments can be trimmed either by entering *Adaptors* (e.g. for **Haloplex** or **Fluidigm**) or by automatically removing sequences at the ends of the fragments. Using the *[Settings]* button the dialogue *Settings* can be opened to adapt settings.

1 Coverage settings

- *Coverage:*
  - *f+r separated:* The coverages in forward and reverse sense are considered separately. A mutation is only listed in the *Variation/Mutation table,* if the coverage reaches the required coverage in forward and reverse direction.
    In case the coverage is regarded separated all absolute values e.g. *Min absolute coverage* are also regarded separated.
    This setting is recommended to use when coverage in sense and antisense is about equal and high enough.
  - *f+r combined:* The coverages in forward and reverse sense are considered combined. A mutation is listed in the *Variation/Mutation table,* if the coverage of the mutation reaches the required coverage compared to the total coverage at the position.
    In case the coverage is regarded combined all absolute values e.g. *Min absolute coverage* are also regarded combined.
    This setting is recommended to use when coverage in sense and antisense is different and not high.
- *Distinct coverage:* If the percentage coverage of a base reaches at least this value the mutation will be listed on tab *distinct* of the *Variation/Mutation table*. The percentage coverage is the coverage of a base divided by the total coverage at this position times 100.
- *Ignore coverage*: If the percentage coverage of a base is below this value the mutation will not be listed in the *Variation/Mutation table*. Therefore all base changed with a coverage below this value are regarded as likely basecalling errors.

  If the percentage coverage of a base is between the *Ignore coverage* and the *Distinct coverage* the mutation will be listed on tab *other* of the *Variation/Mutation table*.
- *Min absolute coverage*: This value refers to the absolute coverage at a position. Sequence-positions with a coverage below this value are written in grey (instead of black). Mutations/variations at those positons are not listed in the *Variation/Mutation table*.

  In case separated coverage is used and the coverage in one sense is below the *Min coverage absolute*, only the sense with the coverage above the *Min coverage absolute* will be regarded. For those mutations the column *Coverage* of the *Variation/Mutation table* is marked pink.

  In case the coverage is regarded separated and there is a mutation in one sense with coverage above the *Min absolute coverage* whereas the other sense is below, the mutation will be marked pink in the *Variation/Mutation table*. This mark shows that the result is based on one sequencing direction only.
- *Min coverage line:* Enter a value for the *Min coverage line*, which is shown as a red dotted line in the electropherogram. In case there are positions with a coverage below this value you will get a warning for the ROI:

- There is the hint *low* in the column *Coverage* of the *ROI/Location table*.
- The graph color of the *coverage graph* (below the *location overview*) changes from grey to pink.

- *set fragments unique*: **Only use this setting for special amplification methods!** For some amplification methods each fragment must have a coverage of only 1. If this setting is active, the coverage of all identical fragments is set to 1.
- *Genome Set:* Select if you use *diploid* or *haploid* DNA.
- *Homopolymer* settings **(only for 454/Ion Torrent data)**:
  - *Min homopolymer*: Default: 40%: Insertions/deletions in homopolymer regions are listed on tab *distinct* of the *Variation/Mutation table* if their percentage coverage is higher than the *Min homopolymer* value. Otherwise they are listed on tab *homopolymer.*
  - *Homopolymer region size:* By default a homopolymer region is defined as at least **five** repeats of a base. This setting can be changed here.

2 Settings to trim or discard fragments

- *compl. fragments only* **(only for 454/Ion Torrent data)**: This setting is only relevant when *Amplicon parts* are defined in the operation *ROIs [master file]* (see chapter 8.3.1.2). Fragments that do not cover the complete *Amplicon part* are discarded.
- *barcode at both ends* **(only for 454/Ion Torrent data)**: Choose this setting in case barcodes have to be present at both ends of the fragments. Fragments with only one barcode are discarded.
- *Remove bases:* Here a number of bases that should be removed at the *5'* or at the *3'* end can be entered into the corresponding field.

Adaptor sequences can be entered to trim or discard fragments (e.g. for **Haloplex** or **Fluidigm**):

- *Adaptor (5'->3')*: Press *[Add]*. Then enter an adaptor sequence in 5'->3' direction. Only active adaptors will be removed. The following fields can be edited for each entered adaptor:
  - *Direction:*
    - *forward:* adaptor is found in forward fragments
    - *reverse:* adaptor is found in reverse fragments
    - *both:* adaptor is found in all fragments
  - *Modifier*:
    - *5'+rev complement*: primer is at the 5' and can also be found rev. complement.
    - *5'*: primer can be found at the 5' end only.
    - *3'+rev complement*: primer is at the 3' end and can also be found rev. complement.
    - *3'*: primer can be found at the 3' end only.
- *Count*: here a value can be entered in case an adaptor is present several times.
- *Error rate*: here a percentage value can be entered as error rate (wrong bases, that the adaptor can contain).
- *Overlap*: Here the minimim number of adaptor bases that must overlap with the fragment can be entered. Example: overlap is *3*. There must be at least *3* adaptor bases found in the fragment.
- box *trimmed/discard*: If *trimmed* is selected the fragment sequences are trimmed (adaptor is cut off). If *discard* is selected all fragments containing an adaptor are discarded.

Moreover there is a function available to automatically remove sequences at the ends of the fragments (e.g. adaptors) that do not match to the reference:

- *Remove ends*: The user can decide to remove bases at the 5' and/or 3'-end by activating the

box. For removing ends a number of bases beginning from the end of the fragment to the number given in the field *Distance* is checked.

- In case there are no mismatches nothing is removed.
- In case there are mismatches it is checked if sequences start to match within the *Distance* area.
  - In case a match is found, the matching sequence must have at least the length entered in the field *Blocklength*. It can include as many mismatches as entered in the box *Max. mismatches*. The bases before (for 5') or behind (for 3') the first matching base are then removed.
  - In case there is no matching block found nothing is removed.

The platform settings are not available any longer, the platform is determined automatically.

1.3.4.3 Tabs

The tab *ROI* comprises the former tabs *PCR Products* and *Enrichment.* Here regions of interest pre-defined in the operation *ROI [master file]* and *ROI Groups [master file]* can be selected.
The tabs *Auto Search Gene* and *Auto Search Genome* are not available any longer.

1.3.4.4 Buttons

The button *[Analyse]* was renamed into *[Start analysis]*.

1.3.5 Operation Joining, Worklist and Archiving /Select 0rders

The search fields *PCR Products* and *Enrichments* are not available any longer, ROIs can be selected in field *ROI.*

1.3.6 Operation Sequence

1.3.6.1 Orders

For old orders (that were not recalculated with version 4.0.0) no settings are displayed.

1.3.6.2 Files, ROI Groups, Genes/Chromosomes, ROIs/Locations

The context menu item *reanalyse* is not available any more.

1.3.6.3 PCR Groups

The dialogue *PCR Groups* was renamed into *ROI Groups*.

1.3.6.4 PCR Products/Locations

The dialogue *PCR Products/Locations* was renamed into *ROIs/Locations*.

New context menu items

- *editing* >
  - *recalculate*: recalculates the location.
  - *settings...*: opens the dialogue *Settings* to change the settings for the location. After

changing the settings the location is recalculated automatically.

- *show* > *ROI Info*: Information about the ROI, such as *Amplicon parts*, *Primers*, *Ignored Sequences* and *Ignored Parts* can be edited.

1.3.6.5 Variation/Mutation table

- The tab *low coverage* is not available any longer.
- A new radio button is availalbe to decide, if mutations are listed for all genes or for the gene selected in the dialogue part *Genes/Chromomosomes* only.
- New column *Gene*, which lists the gene.
- In the column *Pos* the absolute chromosomal postion is listed in parenthesis, behind the position in the reference sequence.
- New mutation type *W* is present, for sequence parts that can not be assigned.

1.3.6.6 Pseudo-Electropherogram and Sequences

1 New layout

The layout of the pseudo-electropherogram/sequences has been improved:

- Defined amplicon parts (operation *ROI [master file]*) are shown as red lines below the Location overview.
- The coverage of the location is shown graphically below the Location overview. In case the coverage is below the *Min coverage line* the graph is marked pink.
- The pseudo-electropherogram is shown for the combined sequence only.
- Bars indicating coverage are shown separated for forward and reverse sense below the pseudo-electropherogram (light blue for forward and purple for reverse).
- Fragment sequences are shown below. The fragments sequences of the fragments in forward and reverse direction are listed. Forward sequences are highlighted in light blue, reverse sequences are written in italics and are highlighted in purple.

  Paired end sequencing data (e.g. **MiSeq** data) are shown as pairs now, overlapping regions are colored orange.

  The number of fragments detected is shown in the beginning of each fragment. For each amplicon/amplicon part only the fragments sequences occurring with the highest coverage are shown. (It can be set in the `lis.ini`-file how many fragments are shown, or if fragments above a certain coverage should be shown). To see all fragment sequences you can open the fragments view as usual.

  The bases can be highlighted in different colors:

  - insertions/deletions have a blue background. Deletions are indicated by a minus sign. Inserted bases are shown, if you move the cursor over the position.
  - mutations listed in the *Variation/Mutations table* have a red background.
  - likely basecalling errors which are not listed in the *Variation/Mutation table* have an orange background.
- When you scroll through a location using the horizontal scroll, the vertical scroll is moved automatically, to show the fragments sequences for the current position.
- Insertions/deletions are shown as red arrows, pointing upwards for insertions and downwards for deletions above the pseudo-electropherogram.

  For a better overview the gene sequence (first green line) is expanded for distinct insertions, there is a - sign at the inserted positions. The distinct insertion is included in forward, reverse and combined sequence.

- Positions with a coverage below the *Min absolute coverage* (can be set ih the operation *Run/Settings*) are visualized in the combined, forward and reverse sequence in the following way by greying out the sequences individually:

  Example 1: coverage in fwd < *MinAbsCov*, but > in rev -> grey out fwd
  Example 2: coverage > *MinAbsCov* in fwd AND rev -> grey out fwd, rev and combined
  Example 3: InDel coverage < *MinAbsCov* in fwd but > in rev -> switch to combined and grey out if fwd + rev < *MinAbsCov*
  Example 4: insertion coverage < *MinAbsCov* in fwd AND rev -> grey out fwd, rev and combined
  Example 5: For heterozygous deletions bases are not greyed out, in case the coverage is below the *Min absolute coverage* for the deleted positions only.

  This visualization supports the fact that a read direction is not considered in the weighting of the base change/insertion/deletion if it is below the *Min absolute coverage*.

2 New mutation type W

A warning is shown for sequence parts that can not be assigned. Those are listed in the *Variation/Mutation table*, the *Type* is *W.* In the pseudo-electropherogram the positions are marked by arrowheads pointing left (for reverse sense) or right (for forward sense). As for all other mutation types warnings are marked red for distinct and grey for not distinct mutations. The sequences can be displayed by opening the context menu (right-click on the arrowhead) and selecting *show fragments view..* or *show Mutation.*

3 Context menu for combined, forward and reverse sequence

There are new context menu items available for combined, forward and reverse sequence:

- *ignore sequence part:* This item can be used to ignore a sequence part. For ignored sequence parts no basecalling is done. Therefore first select a sequence part in the combined, forward or reverse sequence. Then use *ignore sequence part* to ignore this sequence.

  All ignored sequence parts are shown in the operation *ROI [master file]* in the field *Ignored Parts.* For numbering the ignored sequence parts the amplicon count mode is used.

- *copy>:* for all items sequences can be copied in sense or antisense (reverse complement) direction; new item *copy>seq selection>sense/antisense*: copies the selected sequence to the clipboard.

4 Tool tip for combined, forward and reverse sequence

- Quality scores are shown behind each base in the tooltip (only in case they are present in the Next-generation sequencing file) as an alternating colored mark. There are five different colors available, by default dark green means good quality score (close to 100) changing to dark red which means bad quality score (close to 1).
- In the tool tip for the reverse sequence bases are listed complement reverse.

1.3.6.7 Fragments view

- The header of the fragments view now shows the ROI (gene and exon) plus whether it shows the reference or an InsDel. The header is empty if an empty ROI is selected and the fragments view is still open.
- On the new tab *combined* forward and reverse sense fragments are listed. The combined sequence is highlighted light green. Forward sequences are highlighted light blue whereas reverse fragments are highlighted purple. For **MiSeq** data overlapping regions are colored light orange.
- Quality scores are shown when the new field *bases + quality* is selected (only in case they are present in the Next-generation sequencing file). The quality score is visualised as an alternating colored line below each base. There are five different colors available, by default

dark green means good quality score (close to 100) changing to dark red which means bad quality score (close to 1).

- The selected base in the fragments view can be changed with a double left-click on a base. The base position is also changed in the electropherogram then.
- Bases in the fragments view can be marked by pressing Ctrl and mark the sequence using the left mouse button. With a single left-click the selection is removed.
- A sequence can be ignored in the *fragments view* using the context menu entry *save as ignore sequence*. Sequences to ignore can be marked by pressing Ctrl and marking the sequence part with the left mouse button. Sequences ignored here are listed in the field *gnored Sequences/Pseudogenes* in the operation *ROI [master file]*.
- There is the new context menu item *add to filters* available. With this a base can be added to the filter (e.g. a base change, mutation). The new window *Fragments Filter* opens (see below Button *[Filter]*).
- There is the new context menu item *copy* > *sec selection* > *sense/antisense* available to copy a selected sequence in sense or antisense direction. To select a sequence press Ctrl and mark the sequence using your left mouse button.
- Button *[Filter]*: Opens the *Fragment Filter* window. Here you can filter for fragments that fulfill special criteria. When *[OK]* is pressed only those fragments will be displayed in the fragments view. There are several options:
  - mark a position in a fragment in the fragments view and select the context menu item *add to filters*. The *Fragments Filter window* opens automatically. In the table you can select the direction (*fwd, rev, both*) that should be filtered. Moreover you can select *invert* or *normal* in the column *Invert*. With *normal* all fragments that have that base at that position will be shown, with *invert* all fragments that do not that that base will be listed.
  - moreover several options can be activated:
    - *complete paired end*: for paired end data only: only sequence pairs, where fwd and rev sequence is present are shown.
    - *hide BC errors*: fragments with basecalling errors are not displayed.
    - *Quality*: Filter for fragments above a certain quality score (enter 1-100).
    - *Count*: Filter for fragments that are above a certain coverage count.
  - The filter is removed by:
    - right-clicking the entry in the table and pressing *remove*.
    - when the fragments view is closed.
    - when another location/gene and so on... is selected.
- New button *[X/Y scroll]* to switch between two scroll modes for the X and Y scroll: In case this button is pressed the scroll is always moved in X **and** Y direction (default setting). In case the button is not pressed you can scroll in X **or** Y direction separated.
- The button *[All/Matches]* is not available any more. To show matches only, the new filter *hide BC errors* can be used.

1.3.6.8 Report

The report has been worked over.

1.3.7 Operation Pool

In the *Variation/Mutation table* there is a new radio button is availalbe to decide, if mutations are listed for all genes or for the gene selected in the dialogue part *Genes/Chromomosomes* only (like for the operation *Sequence*).

1.4 Module SeqHLA 454 and SeqNext

1.4.1 Operation Sequence

1.4.1.1 Fragments view

- The selected base in the fragments view can be changed with a double left-click on a base. The base position is also changed in the electropherogram then.
- Bases in the fragments view can be marked by pressing Ctrl and mark the sequence using the left mouse button. With a single left-click the selection is removed.

2 Version 4.0.1

2.1 All modules

2.1.1 Locking

When a master file is locked by another user, the name of the user will be listed in addition to the message that the master file is locked.

2.2 Modules SeqPatient, SeqC and SeqNext

2.2.1 Gene Admin

- Genes can be deactivated now. Therefore they are not regarded any more when an autosearch is done. Moreover they are not available any more to create Amp. Modules and Seq Primers and they are not available any more in the *Genes* search fields of *Select Orde*r. To deactivate a gene press *[Extras ->]* and select *deactivate gene*. The mark in the column *Active* of the *Genes table* is then removed. A gene can be activated again by pressing *[Extras->]* and selecting *activate gene*.
- In case several isoforms should be active a hierarchy can be set up. The isoform on top is always the main isoform. To set up a hierarchy for active isoforms use *[Extras->]>change gene*. With the arrows you can change the order of the isoforms.

  When Amp Modules and SeqPrimers are defined the main isoform is used first. For all exons that do not exist in the main isoform the next isoform in the hierarchy is used.

  When loading files and more then one isoform is active and no Amp Modules/Seq Primers are defined, first the loaded files are checked with the first active isoform. If not all sequences can be aligned the second active isoform is checked (and so on). In the *Positions/Resultfiles table* the used isoform can be seen in the column *Loc*. In case not the main isoform is used there is an index behind the location (e.g. "E1 (2)", means that E1 of the second isoform was used).

2.2.2 Operation User

A user who has no right to edit masterfiles can not edit *Gene Admin* as well.

2.3 Modules SeqPatient and SeqHLA

2.3.1 Operation Sequence/Edit Bases

- In *Edit bases* the count modes *AA* (amino acids) and *cDNA* are available now.

- The jumper check > *statistic* is available to jump to positions with statistic warnings in the dialogue *Edit Bases*.

2.4 Module SeqPatient

2.4.1 Operation Joining

Result files with DNA number and defined Seq. Primers that can not be aligned to a gene, can be forced to be joined to an order now. Therefore the entry `JoinRFForced=yes` has to be entered in the `lis.ini`-file, section `[SeqPilot]`. Then result file sequences that can not be aligned are automatically joined to the order depending on the DNA number (for order) and the Seq. Primer (for exon).
Result files that were forced to join have the entry *forced join* in the column *State* of the operation *Joining*.
In the operation *Sequence* those result files are visible in the *Positions/Resultfiles* table and highlighted rose. No sequences are shown since the result file sequence can not be aligned to the gene.

2.4.2 Operation Sequence

For every position/resultfile a comment can be entered in the *Position/Resultfiles* table. Therefore select the context menu item *show* > *comments...*. A new window opens where a comment can be entered. The comment is saved by pressing *[OK]*. Moreover it can be exported as a `txt`-file by pressing *[Save as]*.

2.5 Module SeqNext

2.5.1 Operation Sequence

2.5.1.1 Variation/Mutation table

Base changes and insertions/deletions that are close together (not more than 3 bases in between by default) are regarded as insdels.

Two values can be specified in the `lis.ini`/section `[SeqNext]` to change to regard them as single mutations:

- "`DelInsGapSNPToSNP=`" (default is 3) for the maximum number of bases between two SNPs
- "`DelInsGapSNPToInDel=`" (default is 3) for the maximum number of bases between a SNP and an Insertion/Deletion or between two Insertions/Deletions.

If the gaps between variations are within the thresholds, an InsDel is created which summarizes the variations into one (to be shown as "InsDel" in the mutation table.

2.5.1.2 Alignment

If you want to see all fragments aligned to the reference, even if the alignment is bad (e.g. fragments of a highly mutated virus), you can switch off all quality filters by adding the following entry to the `lis.ini` file: "`UseFilters=no`" (within the "`[SeqNext]`" section).